Structure
Previews

Making the Right Moves

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The structure of the nucleotide-free F_1-ATPase from a thermoalkaliphilic bacterium presented in this issue of *Structure* (Stocker et al., 2007) reveals the structural interactions that prevent the enzyme from operating naturally in the hydrolytic direction. The data provide new insights into the mechanism of the F_oF_1-ATP synthase.

To synthesize ATP at the concentrations necessary to drive many energy-requiring cellular processes, all organisms—from bacteria to humans—utilize the F_oF_1-ATP synthase, a complex membrane-bound multisubunit enzyme. F_o and F_1 refer to the two major components of the enzyme: the integral membrane portion F_o (subunit stoichiometry $ab_2c_{10\text{-}13}$), and the membrane-associated sector F_1 (subunit stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$). The electrochemical proton gradient established by the respiratory chain across the membrane that contains the ATP synthase provides the free energy necessary to drive the ATP concentration to levels $>10^5$ times higher than those expected from the hydrolytic equilibrium ($ATP + H_2O \rightleftharpoons ADP + P_i$).

The mechanism of the ATP synthesis reaction involves large conformational changes in the β subunit in response to the rotation of the smaller γ subunit (Abrahams et al., 1994; Kato-Yamada et al., 1998), driven by proton translocation through the F_o c subunit. The enzyme reaction can also run in the opposite direction to catalyze hydrolysis of ATP. (The isolated F_1 portion of the enzyme, when separated from the membrane, catalyzes only ATP hydrolysis and is called F_1-ATPase.) When the F_oF_1-ATPase runs in the hydrolytic direction, it couples ATP hydrolysis to the generation of a proton gradient across the membrane. This property requires that all organisms have a mechanism to prevent ATP hydrolysis from occurring in situations in which it would be detrimental. Mitochondria rely on a protein inhibitor, IF1. No equivalent protein has been found in chloroplasts or bacteria, but other mechanisms of control over the directionality of the enzyme are operational. In alkaliphilic bacteria, the hydrolytic activity of the ATP synthase is not detectable under most conditions, even with the isolated F_1 portion. It was not until the work of Dimroth and coworkers (Stocker et al., 2007), that this unusual behavior could be understood.

Stocker et al. (2007) report the 3.1 Å resolution structure of the $\alpha_3\beta_3\gamma\varepsilon$ complex from a thermoalkaliphilic bacterium *Bacillus sp.* strain TA2.A1 (TA2F1). As the crystals were obtained without nucleotides, Mg^{2+}, or phosphate in the mother liquor, it is not surprising that the three β subunits are present in the open conformation, corresponding to the empty β subunit (β_E)(Abrahams et al., 1994). In fact, the $\alpha_3\beta_3$ portion of the structure is highly similar to the structure of the nucleotide-free $\alpha_3\beta_3$ structure of the thermophilic bacterium PS3 (Shirakihara et al., 1997) (rms between aligned α carbons = 1.07 Å), which also has all three β subunits in the β_E, open conformation. What is new, then, about the TA2F1 structure? The significant new insights result from analysis of the γ subunit and its interaction with one of the β subunits.

Although the structure of the coiled-coil portion of the γ subunit (γ stalk) of TA2F1 is highly similar to those of *E. coli* and of the mitochondria from yeast, beef heart (Abrahams et al., 1994), and rat liver (Bianchet et al., 1998), its orientation with respect to the $\alpha_3\beta_3$ complex is significantly different: while in the other structures the lower part of the C-terminal helix of the γ stalk is parallel to the approximate 3-fold axis relating the three αβ pairs, the same helix in TA2F1 is tilted by 11° with respect to the axis. This orientation of the γ stalk is stabilized by two adjacent salt bridges formed by the interaction of a cluster of four positively charged residues (Lys8, Arg9, Arg10, and Arg12; Arg9 and Arg10 are directly involved) with two negatively charged residues of the helix-turn-helix (HTH) motif of one of the β subunits (Asp372 and Asp 375). These salt bridges significantly change the interaction of the N terminus of the γ subunit with the HTH motif of that β subunit. The authors argue that these salt bridges are responsible for the absence of hydrolytic activity in TA2F1 (latent ATPase activity). Supporting evidence comes from sequence comparison: bovine mitochondria F1, which has a high ATPase activity, lacks the equivalent of Lys8 in its γ subunit and Asp372 in its β subunit. (Similar sequence changes are found in other active F_1-ATPases.) More convincing evidence is provided by an outstanding experiment: replacement of the KRRIR sequence (residues 8–12) with QQQIQ produced a highly active ATPase. (Note that the mutation produced a "gain of activity" over the wild-type enzyme.) The authors also comment that high ATP concentrations (>2 mM) appear to release the latent ATPase activity, suggesting that "the inhibition is relieved through the binding of Mg^{2+}-ATP and a concomitant conformational change of the corresponding β subunit." The authors further state that the inhibition "would not be affected by conformational changes elicited by nucleotide binding to other β subunits."

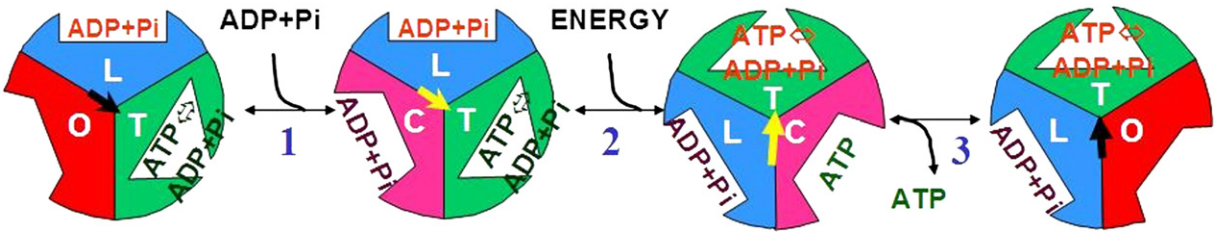

Figure 1. Mechanism of the ATP-Synthase

During ATP synthesis, the reaction proceeds cyclically from left to right through steps 1, 2, and 3. Each circle represents a conformation of the F1-sector of the ATP-synthase. The sectors within each circle represent the three αβ pairs. The conformation of the β subunit in each pair is color-coded: green is tight (T), blue is loose (L), red is open (O), and magenta is closed (C). The substrate(s)/product(s) in the β subunit sites are indicated. Mg^{2+} is not shown. The orientation of the γ subunit is indicated by a short arrow. Before the major rotation (step 2) there are two orientations of the γ subunit distinguished by the color of the arrow: black is the orientation that is present before the three β subunits are occupied (TLO conformation), and yellow is the conformation present when all three β subunits are occupied by nucleotide (TLC conformation). During ATP hydrolysis (i.e., going from right to left), TA2F1 gets trapped in step 3 because the additional double salt bridge present between the γ and the β subunit stabilizes the conformation on the right and prevents the γ subunit to change to the orientation (yellow arrow) that allows closing of the β subunit.

These results do more than provide an explanation for the latent ATPase activity of TA2F1. Why would a salt bridge between the γ subunit and the HTH motif of the β subunit prevent ATP hydrolysis? There is more than enough free energy in the hydrolysis of ATP to overcome this interaction. This means that hydrolysis cannot take place unless some change in conformation occurs when ATP binds to the β_E subunit that is interacting with the γ subunit. Since this ATP mainly interacts with the β subunit, the most likely conformational change is "closing" of the β subunit with a small rotation of the γ subunit in the direction of the major rotation (Bianchet et al., 1998, 2000) (Figure 1, step 3 in the hydrolytic direction, from right to left). (Closing of isolated β subunits upon ATP binding has been observed experimentally; Perez-Hernandez et al., 2002.) The resulting structure would correspond to the three-nucleotide structure observed with the rat-liver mitochondrial F_1-ATPase (Bianchet et al., 1998). Thus, the double salt bridge in TA2F1 makes it harder to close the β subunit upon ATP binding (Figure 1, step 3 going from right to left), blocking the catalytic cycle from continuing to the hydrolytic step. It is clear that increased ATP concentration can partially overcome this blockage by pushing the equilibrium in the direction of more bound ATP (Figure 1, going from right to left).

The results of Dimroth and coworkers (Stocker et al., 2007) strongly support a mechanism in which binding of nucleotide (ATP-Mg^{2+}) to the last empty β subunit changes the subunit from an open (O) to a closed (C) conformation more similar to those of the other subunits with bound nucleotides, the conformations called "tight" (T) and "loose" (L). Bond making (in the synthetic direction) and breaking (in the hydrolytic direction) occurs only with the β subunits in the T, L, and C conformations. By preventing the last β subunit from closing, the double salt bridge in the F_1F_o-ATPase of *Bacillus sp.* TA2.A1 stops the catalytic cycle before ATP hydrolysis can take place. This same interaction does not affect the reaction in the ATP-synthetic direction because the driving force "pushes" the γ subunit in the direction of rotation, breaking the salt bridges and allowing the β subunit to close on the nucleotide before the major conformational change (Figure 1, step1, going from left to right, γ subunit changing from black to yellow).

Thus, the structural and biochemical experiments of Dimroth and coworkers, designed to understand the latent ATPase activity of TA2F1, also provide convincing information about the mechanism of the enzyme. They show that for the last β subunit to bind nucleotide, it has to close around the nucleotide—resulting in an F_1–ATPase in a conformation without an open β subunit—before the complex can undergo the major conformational change involved in energy coupling.

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